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SEXUAL REACTIONS BETWEEN HERMAPHRODITIC AND DIŒCIOUS MUCORS.[1]

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INTRODUCTION.

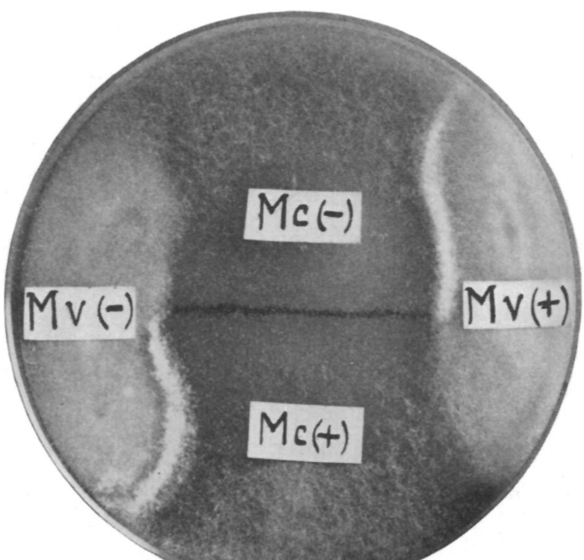
Conjugation among the Mucors has been assumed to represent the simplest type of reproduction acknowledgedly sexual in character. Some even have denied the term sexual to the union of morphologically similar gametes such as occur in this group. The present article will present further evidence in favor of the author's contention that although the process is morphologically a simple one, conjugation in the Mucors is as definitely a sexual process as the morphologically more complex type of reproduction in higher forms and that the sexes seem even more sharply distinct.

It has been shown by the writer ('04, '09) that the majority of the forms among the Mucors are diœcious, with the sexes separated in male and female races which are capable of being propagated apparently to an indefinite number of vegetative generations by means of nonsexual spores formed in sporangia. In all the diœcious species carefully investigated the opposite gametes, which are produced and unite to form zygospores when the two sexual races of a given form are grown together, do not appear to differ morphologically. Lacking a definite criterion which an inequality of the gametes would have afforded, the writer has provisionally designated the opposite sexes in these forms by the signs (+) and (−) on account of a generally greater vegetative luxuriance of one sex over the other. That

[1] Report of investigation carried on, 1912–13, at the Carnegie Station for Experimental Evolution, Cold Spring Harbor, N. Y.

in reality the two sexes are represented in the (+) and (−) groups is shown by the sexual reaction which may occur not only when the (+) and (−) races of the same species are grown together and perfect zygospores are produced, but also by the sexual reaction which may occur when (+) and (−) races belonging to different species are grown together. This reaction between the opposite races of different species has been called "imperfect hybridization" since it does not lead to the formation of perfect hybrid zygospores, but usually stops short with the formation of progametes, though occasionally gametes are produced which, however, never unite.

A sexual race of a diœcious species if grown between the (+) and (−) races of another test species used as a standard, will show a line of imperfect hybridization on one side only. Some



Petri dish culture showing dark line of zygospores between the (+) and (−) strains of a species of *Choanephora* (*MC*) and white lines of "imperfect hybridization" between the strains of this species and the opposite strains of *Mucor V.* (*MV*).

of the hermaphroditic species, on the other hand, when similarly grown, show a response to both (+) and (−) test races and produce therefore two lines of imperfect hybridization. When

the sexual reaction is very strong it may become evident to the naked eye by the formation of a distinct white line as is shown in the adjoining photograph.

Some few species in the hermaphroditic group are distinctly heterogamic with a constant difference in size between the conjugating gametes. It seems reasonable to consider the larger gamete female and the smaller male. Upon this basis, if a sexual reaction could be established between these unequal gametes and the (+) and (−) races, the race reacting with the larger female gamete must be considered male, while the race reacting with the smaller male gamete must be considered female. This was pointed out in 1906 ('06*b*) and in a recent article ('13*b*) it has been shown in brief that sexual reactions have been induced between unisexual races and heterogamic species. The conclusion is reached that the (−) race is to be considered male and the (+) female. The present article will give the detailed results which have formed the basis for this conclusion.

Heterogamic Hermaphrodites.

Since inequality in the size of the gametes in heterogamic species is used as the criterion of sex, it is essential that there be no doubt as to the process of sexual reproduction in these forms. With the exception of *Syncephalis*, which is difficult to cultivate, the heterogamic forms at present known are *Dicranophora fulva* and an undescribed American Dicranophora, neither of which are now in cultivation, *Absidia spinosa*, *Zygorhynchus heterogamus*, *Z. Moelleri* and a number of forms recently described (including *Z. Vuillemini*) which are perhaps too closely allied to *Z. Moelleri* to be deserving of separate names.

The writer in a recent publication ('13*a*) has made a restudy of the genus *Zygorhynchus* and attempted to correct certain misinterpretations of zygospore formation which had been advanced for this genus. Moreau ('12, '13) also, both before and since the publication mentioned, has insisted upon the correct interpretation. So far as concerns the formation and union of unequal gametes, the process is essentially similar to that in *Absidia spinosa* which is the heterogamic species most extensively used in the tests discussed in the present paper.

Absidia spinosa was first found by Lendner and is correctly described by him (Lendner '08). Its zygospores agree with other Absidias in possessing circinate outgrowths which develop from the suspensor soon after the formation of gametes. It differs from other Absidias, which it resembles in its method of nonsexual multiplication, by being hermaphroditic (cf. right-hand side of Fig. 8, Plate I.) and heterogamic (Fig. 4). Figs. 1–7 represent stages in development in living material drawn at the times indicated in the legend. A considerable number of conjugations have been followed in living material and stages have been studied in stained and mounted material. Unequal gametes, such as are shown in Fig. 4, unite, as in Fig. 6, and the zygote thus formed grows into a mature zygospore such as is shown in Fig. 7. In moist chamber cultures, drops of fluid generally accumulate around the uniting gametes (Fig. 6), often causing appearance of transverse lines that suggest additional cross walls. They can be distinguished from cross walls, however, by forcing the conjugating filaments against condensed moisture on the under side of the cover-glass. Perhaps the most typical form of conjugation is that shown in Fig. 8, where a rather stout branch on the right has applied itself to the more delicate termination of the main axis causing the production from the latter of a small male gamete, while it produces from its own enlarged tip a large female gamete. Curved outgrowths arise from the swollen suspensor but are not produced from the side of the smaller gamete. In *Zygorhynchus* a septum is regularly formed across the main axis just above the origin of the stout conjugative branch. This septum is as regularly absent in *Absidia spinosa*.

When for any reason the process of conjugation is arrested after the formation but before the union of gametes, these sexual cells may round themselves off, form thick walls and become azygospores. This is brought about in greater or less degree when the conjugating filaments in a moist chamber culture become immersed in a drop of condensed water on the underside of the cover-glass. Whatever the cause of the check to normal conjugation, the size of the azygospores depends upon the size of the gametes from which they develop. Thus in Fig. 33 it

would be possible to label the azygospores of *A. spinosa* male and female, from their relative size alone, even if one were not subtended by a larger suspensor and surrounded by the outgrowths peculiar to the side of the female gamete. Similarly *Zygorhynchus* may produce male and female azygospores from adjacent gametes that have failed to fuse (Fig. 13). As will be shown later, azygospores may be produced from gametes that have formed as the result of a sexual reaction with a different species. If the opposite gametes of hermaphroditic forms are strictly male and female, it would theoretically be possible to transform a hermaphroditic species into male and female races by germinating its male and female azygospores. This has been attempted ('06*a*) and germinations have been obtained of the azygospores of *Sporodinia*. The mycelia that have thus been obtained produced only a very scanty growth and soon died out before it was possible to test their sexual reaction. Germinations of other azygospores, so far as the writer is aware, have never been critically investigated.

Homogamic Hermaphrodites.

Spinellus fusiger, *Sporodinia grandis*—two forms found upon fleshy fungi,—*Mortierella polycephala* and *Mucor Genevensis*—are the only homogamic forms definitely known among hermaphroditic Mucors. In none of these is there apparently a constant difference in the size of the gametes. Neither *Spinellus* (which is difficult to cultivate on artificial media) nor *Sporodinia* shows sexual reactions with other species. *Mortierella* has not been available for study. *M. Genevensis*, of which the writer has races from four distinct sources as well as derived mutants, shows a strong sexual reaction with both (+) and (−) sexes of diœcious species. The reaction has already been noted ('04*b*, p. 311, pl. IV., fig. 56). Observation on living material shows the reaction to be similar to imperfect hybridization between different diœcious species. The formation of azygospores, however, has been observed in different races of this hemaphroditic species as a result of the stimulus of contact with (+) and (−) strains of Mucor V. Azygospore formation will be further discussed under *Absidia spinosa* below.

Diœcious Species.

No diœcious form of the Mucors is known to be regularly heterogamic. In *Rhizopus* ('04*b*, Fig. 15) and in other diœcious species the writer has found that the inequality in size between conjugating gametes has no necessary relation to sex, since the larger gamete is formed sometimes by one sex, and sometimes by the other. In many species, however, the (+) race regularly shows a decidedly greater vegetative vigor than its (−) mate. This greater vigor of growth may be responsible for the fact that the conjugative filaments arising from the (+) mycelium average stouter than those from the (−), and this difference may be accompanied by a similar difference in the gametes and suspensors. One was generally able to recognize the (+) from the (−) sides of the line between the two sexes of Mucor V. by this greater vigor of the (+) conjugative filaments but even in this form where the distinction is most marked, the stouter of the conjugating filaments is not invariably on the (+) side. Mucor V. is a form found by the writer ('04*b*) in 1904. It is apparently not specifically distinct from *M. hiemalis* Wehmer, the sexual races of which were isolated by Hagem ('08) and forms zygospores, though in no great abundance, with the strains of this name sent from the "Centralstelle" at Amsterdam. It differed from the Amsterdam material, however, in its much greater sexual vigor. In 1912 when the reactions described in the present paper were investigated, the Amsterdam *M. hiemalis* failed to show any reactions with other species tested, while Mucor V. was sexually the most active of all the Mucors known. At the present writing, however, Mucor V. has apparently become weakened in sexual activity. Hagem ('08) reports a similar loss of sexual activity in one of his strains of *M. hiemalis*.

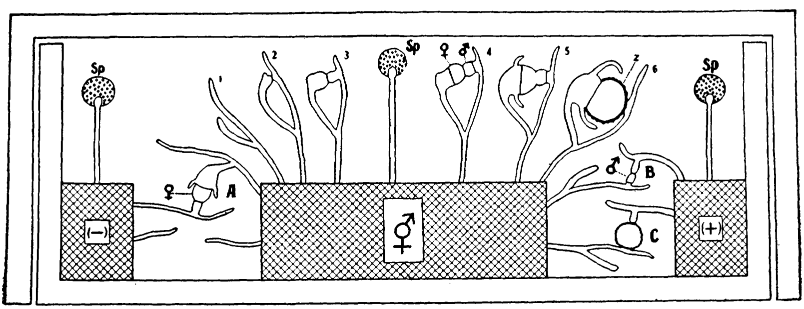
Reactions between Diœcious Species and Absidia spinosa.

The difficulties in technique involved in following the sexual reactions in a thicket of filaments have been overcome by growing the heterogamic hermaphrodite (☿) in a Petri dish between the (+) and (−) test strains and cutting channels in the nutrient agar between the different growths. If the Petri dish be

then inverted, the growth of the reacting filaments may be followed in mid air in the channels, under the low power objective.

Absidia spinosa is the only heterogamic species which has been found to give reactions with both (+) and (−) races and Mucor V. is the only diœcious form that will react with both male and female gametes of *A. spinosa*. These have accordingly been most extensively employed in making the tests shown in the accompanying diagram.

In the left-hand channel at *A* in the diagram, a filament from the (−) race is shown giving a sexual reaction with the larger ♀ gamete of the hermaphrodite, while in the right-hand channel at *B* a filament of the (+) race is figured, showing a sexual reaction with the smaller ♂ gamete of the hermaphrodite. The



Diagrammatic representation of a Petri dish culture showing a heterogamic hermaphroditic mucor (⚥) in the center separated by channels on either side from the (+) and (−) races, respectively, of a diœcious species.

Sp., Sporangia containing spores by means of which the plant may be reproduced nonsexually.

1–6, stages in development of a hermaphroditic zygospore from unequal male and female gametes.

A, sexual reaction between a (−) filament and female gamete.

B, sexual reaction between a (+) filament and male gamete.

C, a male azygospore formed at stimulus of contact with a (+) filament.

male gamete, which has been cut off from a filament of the hermaphrodite at the stimulus of contact with a (+) hypha, frequently surrounds itself with a thick wall and assumes the appearance of a resting azygospore, as is shown at *C*.

Figs. 20–24, Plate II., are drawings from living material. The female gamete of *A. spinosa* is recognized not only by its

larger size but also by the curved outgrowths that arise from its suspensor. It will be observed further from these figures that the female gamete is formed from a rather stout branch which appears to have been attracted by Mucor V. (−) and drawn away from the delicate termination of the main axis with which it would normally have conjugated (cf. Fig. 8). In order to obtain such reactions as shown in Figs. 20–24, some care had to be exercised in regard to the condition of the culture. If the nutrient is too favorable for growth the filaments will choke the channels and cannot be followed. If the nutrient is deficient, conjugations will not occur. The depth and width of the channels and the time at which they are cut must be carefully regulated or no reactions can be observed. They have been obtained and studied in a sufficient number of cases, however, to leave no doubt as to their occurrence. Mucor V. is the only form whose (−) race was found to give a sexual reaction with *A. spinosa*.

The sexual reaction of *A. spinosa* with the (+) race of Mucor V. is entirely different from what has just been described. At the line of contact between the two mycelia, the filaments are much branched and imperfect hybridizations are abundant. Figs. 18 and 19 show simplifications of this condition from living material. Fig. 17 shows gametes cut off from *A. spinosa*, while Figs. 14–16 show such gametes transformed into dark thick-walled azygospores. Fig. 15, which shows the progamete of Mucor V. (+) rounded off and in bare contact with the azygospore of *A. spinosa*, is a typical condition.

The (+) race of *Absidia cærulea* (*A* (+), Figs. 8–10) shows a less active sexual reaction with *A. spinosa* but similar to that shown by Mucor V. (+), and the same is true of *A. cylindrospora* and Mucor N. The (+) strains of all four diœcious species mentioned are capable of stimulating the production of small male gametes from filaments of *A. spinosa* and their transformance into dark thick-walled azygospores. Unfortunately it has not yet been possible to bring these azygospores to germination. It would be interesting to discover if their germinations would give rise to unisexual male mycelia.

Judging from the reactions just described between *Absidia*

spinosa and the sexual races of Mucor V., one would seem justified in considering the vegetatively more vigorous (+) race as female, and the less vigorous (−) race as male. The terms male and female therefore may be used hereafter in distinguishing the sexual races of diœcious Mucors.

Reactions between Diœcious Species and Zygorhynchus.

Zygorhynchus Moelleri shows no sexual reaction with the (−) race of Mucor V. but contact with its (+) race causes the production of delicate, contorted, warty filaments which take part in imperfect hybridizations as shown in Fig. 12. The gametes formed by *Z. Moelleri* in contact with Mucor V. (+), frequently are transformed into azygospores shown in Fig. 11. *Z. Vuillemini* apparently is identical with *Z. Moelleri*, at least so far as its sexual reactions with Mucor V. are concerned.

In contrast with these two Zygorhynchus forms just discussed which show only a (−) or male tendency in contact with Mucor V., *Z. heterogamus* gives evidence of a female tendency. Its reactions with the (−) race of Mucor V. are shown in Figs. 25–28. It does not seem to react with (+) races and even with the (−) race it is not easy to induce reactions and those that result do not lead to azygospore formation. If Mucor V. (−) in Fig. 25 is actually showing a sexual reaction with the male gamete of *Z. heterogamus* as might appear from its position on the terminal filament of the latter, the evidence would be in conflict with that to be drawn from the reactions with *A. spinosa*, *i. e.*, that the (−) race is male and the (+) race female. It will not be possible to attach much significance to the individual reactions in *Zygorhynchus*, however, since so far no form of this genus has been induced to react with more than a single sexual strain of the test diœcious species.

Z. Vuillemini var. *agamus* is a zygosporeless strain which Namyslowski (10) separated from a culture of *Z. Vuillemini*. It was found impossible to induce zygospore formation from cultures of this species supplied by the Centralstelle. In contact with the (+) race of Mucor V. (Figs. 30–32) however, it reacted like the sexually active strain of *Z. Vuillemini* and even produced azygospores (Fig. 32). This form therefore has a male tendency.

Judged by their reaction with the (+) and (−) strains of Mucor V., the forms *Z. Moelleri*, *Z. Vuillemini* and *Z. Vuillemini agamus* have a male tendency, while *Z. heterogamus* has a female tendency. It is in harmony with this classification that *Z. Vuillemini agamus* produces azygospores in contact with *Z. heterogamus* (Fig. 29).

Summary

It has been shown that sexual reactions called "imperfect hybridization" may occur when hermaphroditic species of Mucors are grown in contact with the sexual races of diœcious forms. Certain of these hermaphrodites are heterogamic, showing a constant difference in the size of their gametes. It is assumed that the larger gamete is female and the smaller one male. The race of diœcious Mucors provisionally called (+) shows a sexual reaction with the smaller or male gamete while the (−) or vegetatively less vigorous race shows a reaction with the larger or female gamete. It is concluded therefore that the (+) race of diœcious Mocurs is female and the (−) race, male.

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EXPLANATION OF PLATES.

All figures were outlined with the aid of a camera lucida. Those in outline were taken from stained and mounted material. Those stippled show development in living material from moist chamber cultures. Figs. 8–12, 18–24 and 31–32 were viewed with a low power objective and were magnified by the use of increased tube length or higher eye-pieces. Other figures represent high power drawings.

Plate I.

Figs. 1–7. *Absidia spinosa.* Consecutive stages in zygospore development from living material. Only those outgrowths in median focus are represented. Drawings were made at following hours: Fig. 1 at 4.15 P.M., Dec. 4; Fig. 2 at 4.52 P.M.; Fig. 3 at 5.50 P.M.; Fig. 4 at 7.10 P.M.; Fig. 5 at 12.30 A.M., Dec. 5; Fig. 6 at 2.15 A.M.; Fig. 7 at 3.00 P.M. The dotted circle in Fig. 6 represents the outline of a drop of fluid around the young zygote.

Figs. 8–10. "Imperfect hybridization" between *Absidia spinosa* (☿) and the (+) race of *Absidia cærulea* (*A*(+)). Fig. 8 on right, typical conjugation of *Absidia spinosa;* on left, "imperfect hybridization." Fig. 9 drawn at 8.30 A.M., Sept. 23. Fig. 10 drawn at 4.00 P.M., Sept. 24. Note azygospore formed from the male gamete of the ☿ hypha.

Figs. 11 and 12. "Imperfect hybridization" between *Zygorhynchus Moelleri* (☿) and *Mucor V.* (+). Fig. 11, male azygospore from ☿ hypha. Fig. 12, three "imperfect hybridizations."

Fig. 13. Azygospores formed from male and female gametes of *Zygorhynchus.*

Figs. 14–17. "Imperfect hybridizations" between *Mucor V.* (+) and *A. spinosa* (☿). Figs. 14–16, azygospores developed from male gametes of the ☿. Fig. 17, gametes on the ☿.

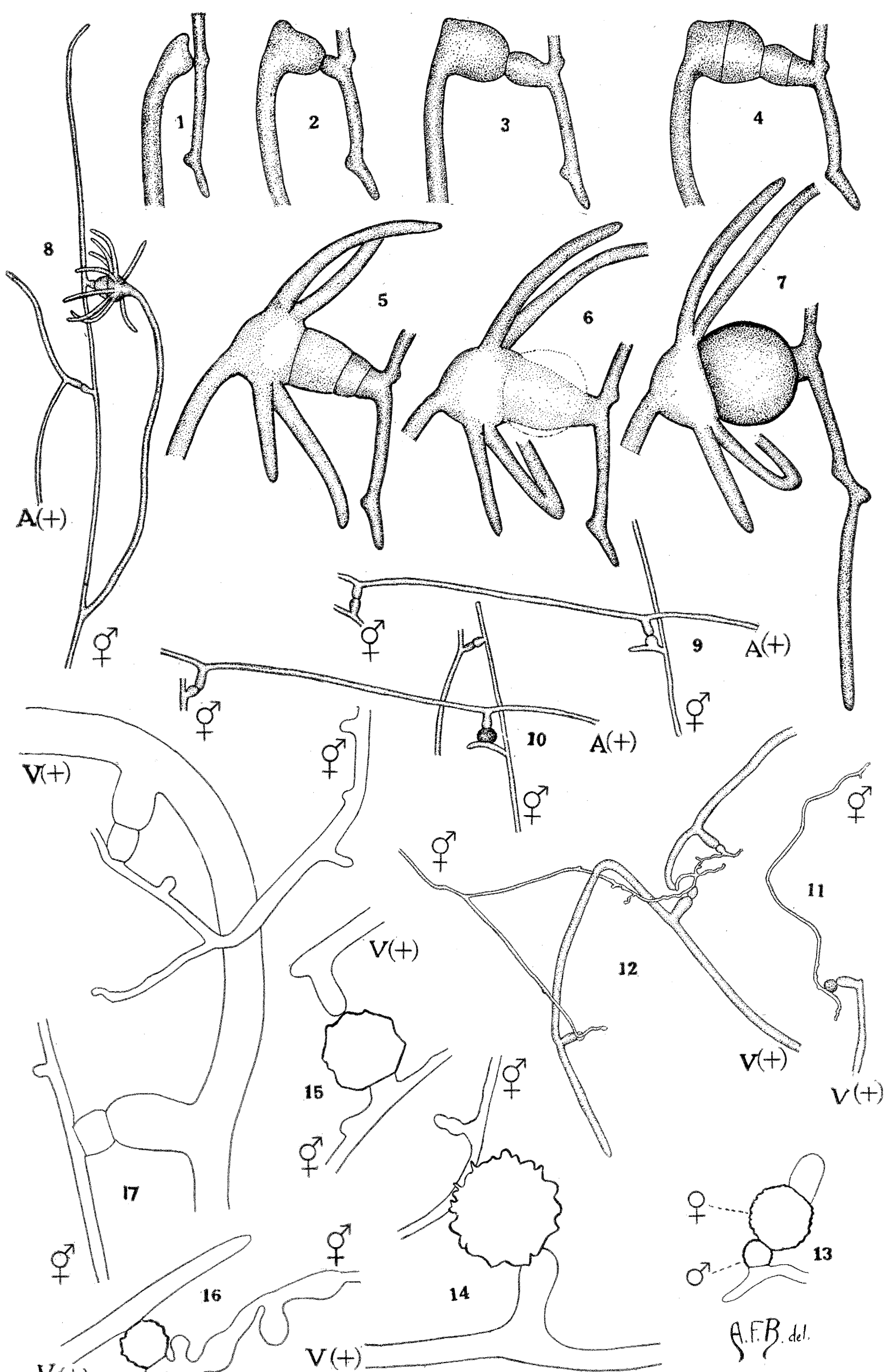

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Plate II.

Figs. 18 and 19. "Imperfect hybridizations" between *Mucor V.* (+) and *A. spinosa* (☿) drawn from living material.

Figs. 20–24. "Imperfect hybridizations" between *Mucor V.* (−) and *A. spinosa* (☿). Fig. 20 drawn at 7.30 A.M. Fig. 21, the same at 3.30 P.M., Sept. 12. Fig. 23 drawn P.M., Sept. 12. Fig. 24, the same, A.M., Sept. 13.

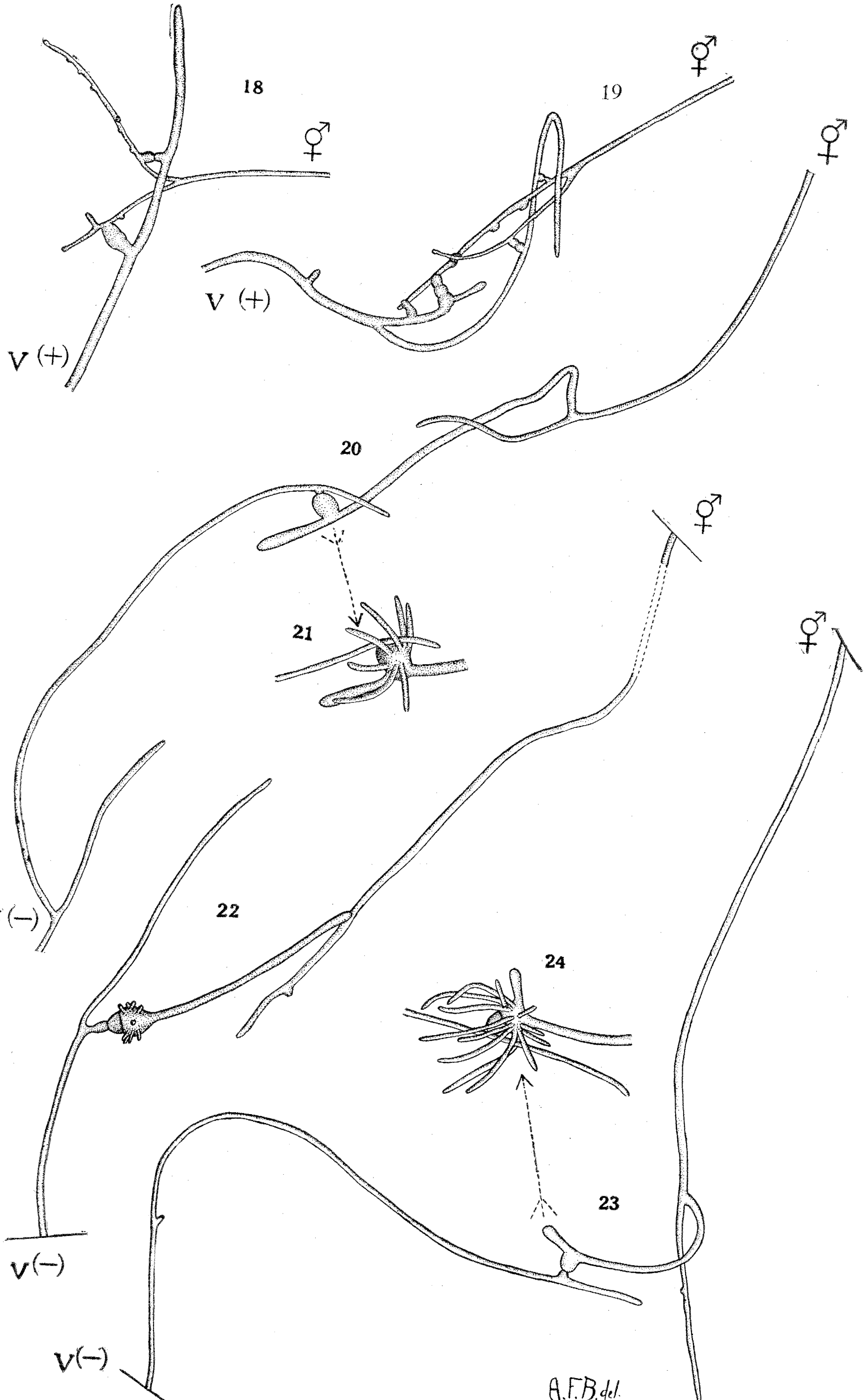

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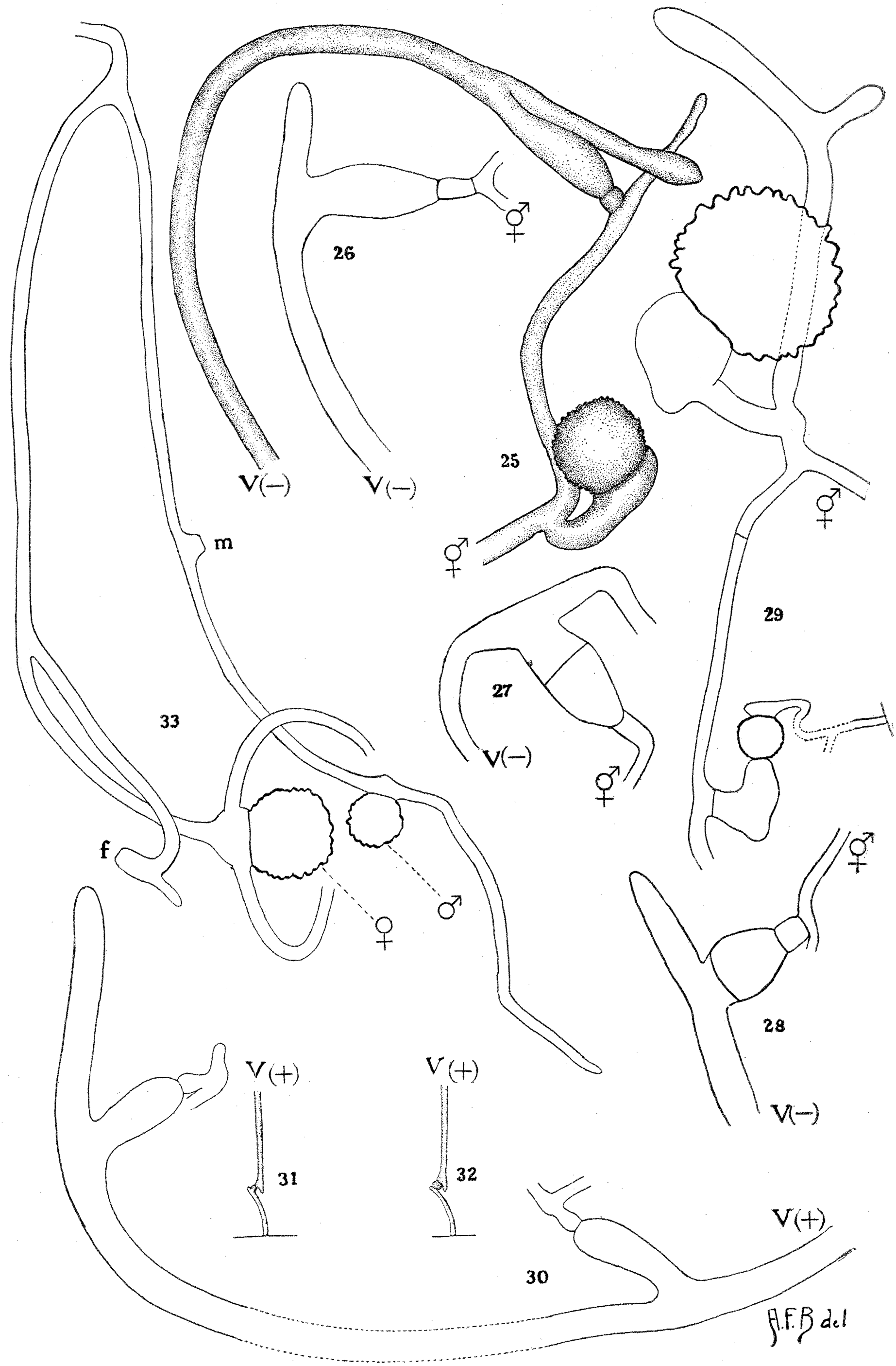
Plate III.

Figs. 25–28. "Imperfect hybridizations" between *Mucor V.* (−) and *Zygorhynchus heterogamus* (☿). Fig. 25 from living material. Figs. 26–28, gamete formation.

Fig. 29. "Imperfect hybridization" between *Zygorhynchus Vuillemini* var. *agamus* and *Zygorhynchus heterogamus* showing formation of azygospore from gamete of former species.

Figs. 30–32. "Imperfect hybridizations" between *Mucor V.* (+) and *Zygorhynchus Vuillemini* var. *agamus*. Fig. 30, progamete formation. Fig. 31 drawn at 9.55 P.M., Dec. 20. Fig. 32 drawn at 10.45 A.M., Dec. 21.

Fig. 33. Apposed azygospores of *Absidia spinosa* developed from male and female gametes. *m* and *f* apparently represent respectively male and female progametes which have been pulled apart in mounting.



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